

X-723-66-155

NASA TM X-55520

BIOLOGICAL DECONTAMINATION OF A SPACECRAFT SYSTEM

BY

GPO PRICE \$ _____

F. N. LeDOUX

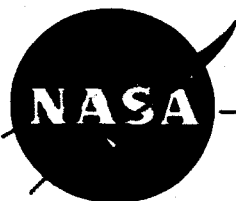
CFSTI PRICE(S) \$ _____

Hard copy (HC) 1.00

Microfiche (MF) 50

653 July 65

APRIL 1966



GODDARD SPACE FLIGHT CENTER
GREENBELT, MARYLAND

N66 30343

(ACCESSION NUMBER)

21
(PAGES)

TMX-55528
(NASA CR OR TMX OR AD NUMBER)

(THRU)

(CODE)

31
(CATEGORY)

FACILITY FORM 802

X-723-66-155

BIOLOGICAL DECONTAMINATION OF A SPACECRAFT SYSTEM

by

F. N. LeDoux

Goddard Space Flight Center
Greenbelt, Maryland

Paper presented March 31, 1966 at Houston, Texas to American Association
for Contamination Control

BIOLOGICAL DECONTAMINATION OF A SPACECRAFT SYSTEM

by

F. N. LeDoux

ABSTRACT

30393

The NASA Office of Planetary Quarantine, requires biological decontamination of all spacecraft for missions near the moon.

Before such program was started the following determinations were made.

A 15 second brush-wash with xylene and rinse in isopropyl alcohol removed 100% of rosen flux deposits of from 73 to 113 milligrams. Kill effectiveness tests indicated that a 95% kill could be obtained by immersing components in a 90% isopropyl alcohol for 15 minute period. Xylene and alcohol were both found to be compatible with the electronic circuits and spacecraft materials.

To determine the effectiveness of decontamination, two methods of recovering viable organisms were employed. Control strips with detachable coupons were used for recovery from the electronic circuit modules, and sterile swabs and templates were used for other surfaces.

Coupons were washed in a 1% peptone solution for 15 minutes, and pour plates were prepared using aliquots of the solution. Tryptic soy agar was the nutrient. The pour plates were incubated at 32°C for 72 hours and then plate counts were made. The contaminated swabs were placed into sterile water; pour plates were prepared, and incubated, and counts made.

The greatest source of contamination is probably the technicians themselves and the debris generated during mechanical integration and/or assembly. To minimize this a clean room complex was built that allowed for decontamination of spacecraft components, taking samples for assaying, and final assembly.

The total viable count is determined by adding the counts for different areas at the time of occlusion, the counts for all interior and exterior surfaces, and an estimate of the internal burden of components.

Author

BIOLOGICAL DECONTAMINATION OF A SPACECRAFT SYSTEM

Because of the harsh environment of the moon, it is assumed that any contamination of the lunar surface by a spacecraft will remain localized and will not propagate significantly. On this basis it was determined by the Office of Planetary Quarantine, NASA Headquarters, that complete sterilization of spacecraft would not be necessary for lunar missions. However, they do require that such spacecraft be biologically decontaminated to a low level of viable organisms at time of launch.

For the purpose of this paper biological decontamination is defined as: the killing and/or removal of the greatest possible number of viable microorganisms which are capable of independent existence, and the removal of all other residuals which may serve as nutrients to support microbial life. When components can withstand the sterilization environment in an autoclave or dry heat sterilization temperatures without affecting the reliability of a system, they will be sterilized. In all other cases biological decontamination shall mean sterilization of as many surface areas as possible.

Before a program for biological decontamination of spacecraft systems was inaugurated, several determinations had to be made.

- a. Effectiveness of flux solvents.
- b. Agents for bacteriacidal action.
- c. Compatability of spacecraft materials with bacteriacide.
- d. Method of recovering viable microorganisms from surfaces.
- e. Means for enumerating the probable total viable load at time of launch.
- f. Means of asepsis handling, assaying and final assembly.

First it was desired to determine which of several solvents would serve best as a cleaner of residual flux deposits and other foreign materials present on the electronic instrumentation modules. An investigation

was conducted in which four different solvents were evaluated. Two of these were eliminated early in the testing program because of factors other than cleaning efficiency.

Although the solvents eliminated were adequate from a cleaning standpoint they caused a rapid deterioration of the rubber gloves used by the technicians in applying the solvents. It is recognized that both Xylene and Moore-50 solvents caused some deterioration of the gloves used but, less rapidly.

Figure 1 is a summary of results of tests conducted using Xylene and Moore-50 solvent. These solvents were used in conjunction with resin fluxes extracted from:

1. Ersin Multicore Solder
2. Kester Core Solder (No. 66)
3. Kester Plastic Core Solder

FLUX SOLVENT EFFICIENCY TEST RESULTS

FLUX			FLUX CLEANING TIME WITH SOLVENT (SECONDS)	AVERAGE EFFECTIVENESS OF CLEANING %
CLEANING SOLVENT	TYPE	AVERAGE MASS IN MG		
XYLENE ↓	ERSIN MULTICORE	113	15	100
	KESTER CORE #66	119	15	100
	KESTER PLASTIC CORE	73	15	100
MOORE M-50 ↓	ERSIN MULTICORE	98	15	99.8
	KESTER CORE #66	87	15	99.7
	KESTER PLASTIC CORE	87	15	100

Figure 1.

The average mass of flux deposits used in conducting the solvent efficiency tests was 96 milligrams. The average amount is approximately four times that which would be expected to be found on a circuit connection. The cleaning time of 15 seconds was maintained constant for each of these reported tests.

Next a determination was made for achieving microbial decontamination employing a decontaminating agent compatible with the electronic components, conformal coating, and encapsulation material. Past experience and tests on coupons from control strips indicated that complete immersion and agitation of a module in 90% isopropyl alcohol for at least 15 minutes was a practical method of killing and removing most vegative cells, and at the same time remove entrapped moisture that could carry nutrients. Filtering the used isopropyl alcohol showed that viable spores were washed free of the surface of electronic components. It was realized that the free spores in the alcohol could contaminate other items being decontaminated, therefore, a filter system was set up that would filter out particles .5 μ in diameter and larger. Figure 2 shows the system used to filter the spore contaminated alcohol.

Before reusing any of the propanol for decontaminating a circuit module it was first run through the filter system for a period of five minutes. In this manner freshly filtered propanol was used for each decontamination.

The next determination that had to be made concerned the compatability of spacecraft components with the xylene and isopropyl alcohol that would be used as the decontaminating agents. Figure 3 is a summary of tests conducted on electronic components.

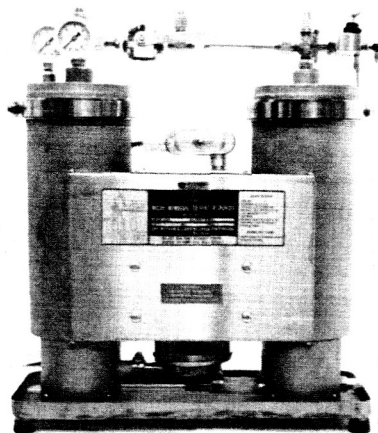


Figure 2.

SUMMARY OF TESTS & RESULTS ON ELECTRONIC COMPONENTS.

UNIT NOMENCLATURE	NO. OF UNITS.	EFFECTS OF TREATMENTS ON ELECTRICAL PROPERTIES						REMARKS
		XYLENE	PROPANOL	CONFORMAL COAT	POTTING	HEAT (1) SOAK	COLD (2) SOAK	TEMPERATURE CYCLE (3)
IMP MULTICONVERTER	1	NONE	NONE	NONE	—	NONE	NONE	NOISY ZENER DIODE
AUDIO FILTER	2	NONE	NONE	NONE	NONE	NONE	NONE	Overall frequency shift on filters was less than one percent: still flightworthy.
S-049 INCREMEG DECADE COUNTER	1	NONE	NONE	NONE	—	—	—	
2-BIT BINARY CIRCUIT	2	NONE	NONE	NONE	NONE	NONE	NONE	
100 KC AMPIFIER	1	NONE	NONE	NONE	NONE	NONE	NONE*	FAILED*
S-060 INCREMEG DECADE COUNTER	2	NONE	NONE	NONE	NONE	NONE	NONE	NONE
S-496 INCREMEG DECADE COUNTER	1	NONE	NONE	NONE	NONE	NONE	NONE	NONE

- Notes: (1) Heat soak = held at 65° C for 48 hours.
 (2) Cold soak = held at -60° C for 48 hours by means of CO₂ atmosphere.
 (3) Temperature cycle = 3 hours at +65° C, 1 hour transition time to hold at -60° C for 3 hours, remove and let return to ambient.

• Cold soak temperature & lower temperature of cycle changed to -65° C for this component only.

Figure 3.

The electronic circuits used in conducting these tests were first electrically checked by the cognizant experimenter before any treatment.

After each treatment i.e., cleaning with xylene - decontaminating with propanol prior to conformal coating and for encapsulation they were again re-checked so as to determine the effects on the circuits by the treatment. Environmental tests were then performed on each of the circuits indicated. Heat soak at 65°C for 48 hours and a cold soak at -60°C for another 48 hours. The circuits were again rechecked after each treatment. They were then subjected to a temperature cycle i.e., 3 hours soak at 65°C with a 1 hour transition to -60°C and soaked at this temperature for 3 hours before returning to ambient room temperature.

The results indicated a frequency shift less than 1% on the audio filter circuit, however, this component was considered flightworthy.

A noisy zener diode was noted when checking the multiconverter after temperature cycle. However, the multiconverter used in conducting the test was used for over a year as a laboratory work-horse. The development of the noisy diode could have been due to its repeated use. There is no evidence to substantiate that the failure occurred as a result of the decontamination process. In the future it is advisable to conduct the environmental tests first.

Figure 4 data is based upon a limited number of tests. The average tensile shear strength of the unexposed samples averaged 1285 P.S.I. and was based upon testing 5 samples. Only 2 samples were tested that were first exposed to a 1/2 hour soak in propanol and agitation. The increase in shear stress in

NOMENCLATURE	AVERAGE TENSILE SHEAR STRESS P.S.I.	
	UNEXPOSED	1/2 HOUR SOAK PROPANOL
HYSOL 1-C	1285	1370
TWIN-WELD	1520	1690
EPON 828	962	740
BIGGS 823 MODIFIED	952	1177

Figure 4.

Hysol 1-C, twin-weld and the Biggs 823 modified may have occurred due to a more thorough cleaning action of the propanol. However, more tests should be conducted on a greater number of samples so as to obtain more meaningful data. The reduction in shear strength of the Epon 828 was within design limits but this material will not be used in any critical area in the spacecraft.

Samples of all other structural materials that were to be used in the spacecraft were checked for surface microstructure and reflectivity. They were then subjected to a 1/2 hour soak and agitation in propanol. Tests were then conducted so as to determine changes in surface microstructure and/or reflectivity due to the propanol decontamination. Micro examinations were made at 1200X magnification. The reflectivity tests were made over the solar spectrum. There was no change noted in any of the samples tested.

These tests, shown in Figure 5, were conducted by the Thermal System Branch personnel at Goddard.

THERMAL COATINGS ABSORPTIVITY TESTS THERMAL SYSTEMS BRANCH

ABSOLUTE DEVIATION FROM CONTROL SPECIMEN CHARACTERISTICS		
COATING DESCRIPTION	EFFECTS ON ABSORPTIVITY	
	PROPANOL	ACETONE
DOW CORNING, METHYL SILICONE TiO_2	$\Delta \alpha = .02$ DECREASE	$\Delta \alpha = .006$ DECREASE
CAT-A-LAC BLACK	$\Delta \alpha = 0$	$\Delta \alpha = .006$ DECREASE
VAPOR DEPOSITED ALUMINUM	$\Delta \alpha = .031$ INCREASE	$\Delta \alpha = .004$ INCREASE

NOTE: Absorptivity measurements conducted over entire solar spectrum .3 - 2.5 μ .

Figure 5.

This summary is based on the absolute differences in absorptivity of light waves, which occurred between a non-decontaminated control specimen and test specimens which were decontaminated with propanol or acetone.

The change in absorptivity using propanol was considered as significant. The acetone was considered to be the most desirable; however, when applying either of the solvents to white painted surfaces there was a decrease in solar absorptance. With this change in mind the Thermal System Branch test conductor recommended that only the buffed and black surfaces be 100% decontaminated. These surfaces constitute over 85% of the total exposed area of the spacecraft.

To determine the effectiveness of spacecraft decontamination. Two methods of recovering remaining viable organisms from the surfaces of decontaminated areas and components were employed. One method employed control strips with detachable coupons to recover organisms from the electronic circuit module frames. Figure 6 shows such a control strip affixed to a frame.

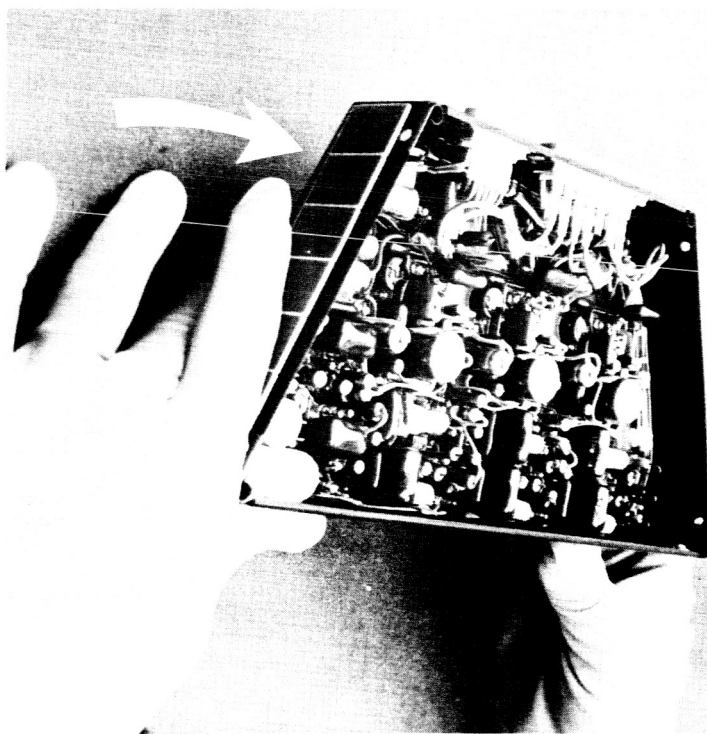


Figure 6.

Control strips were affixed to each module frame in a similar manner, so located as to compel the technician to touch the control strip each time he handled the module, thus creating the worst condition for contamination. The purpose of the control strip is to prove the effectiveness of the decontaminant and to allow for a practical method for enumerating probable viable organisms remaining or components at each stage of occlusion. These control strips were fabricated in such a manner as to yield five easily removed coupons of the same material as the printed circuit boards. After each decontamination—such as prior to conformal coating, encapsulation, integration, and final assembly—a coupon was removed and assayed for count of viable organisms. Each item decontaminated and its control strip were handled in the same manner and at the same time. Coupons were placed into a wash bottle containing 15 ml of a 1% solution of sterile peptone. Wash bottles were then shaken with a wristaction motion for 5 minutes before transferring aliquots to pour plates. Two pour plates were so prepared, each containing a 5 ml aliquot of the contaminated wash solution. In addition, pour plates were prepared using a 20 ml each of sterile tryptic soy agar as the nutrient. All of the plates were incubated at 32°C for a period of 72 hours and then plate counts were made.

The second method of recovering viable organisms employed sterile swab and template to sample surface areas prior to their occlusion by attachments. After swabbing the appropriate surface each swab was placed in a tube containing 10 ml of sterile distilled water. Each tube was mechanically shaken for 5 minutes. After shaking 4 ml aliquots were plated in duplicate and colony counts made. Figure 7 shows such a recovery operation.

The next three figures depict the manner in which the total viable count for a spacecraft system is being arrived at.

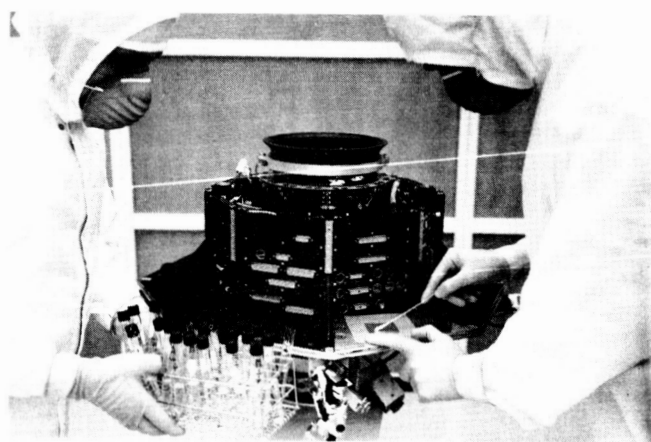
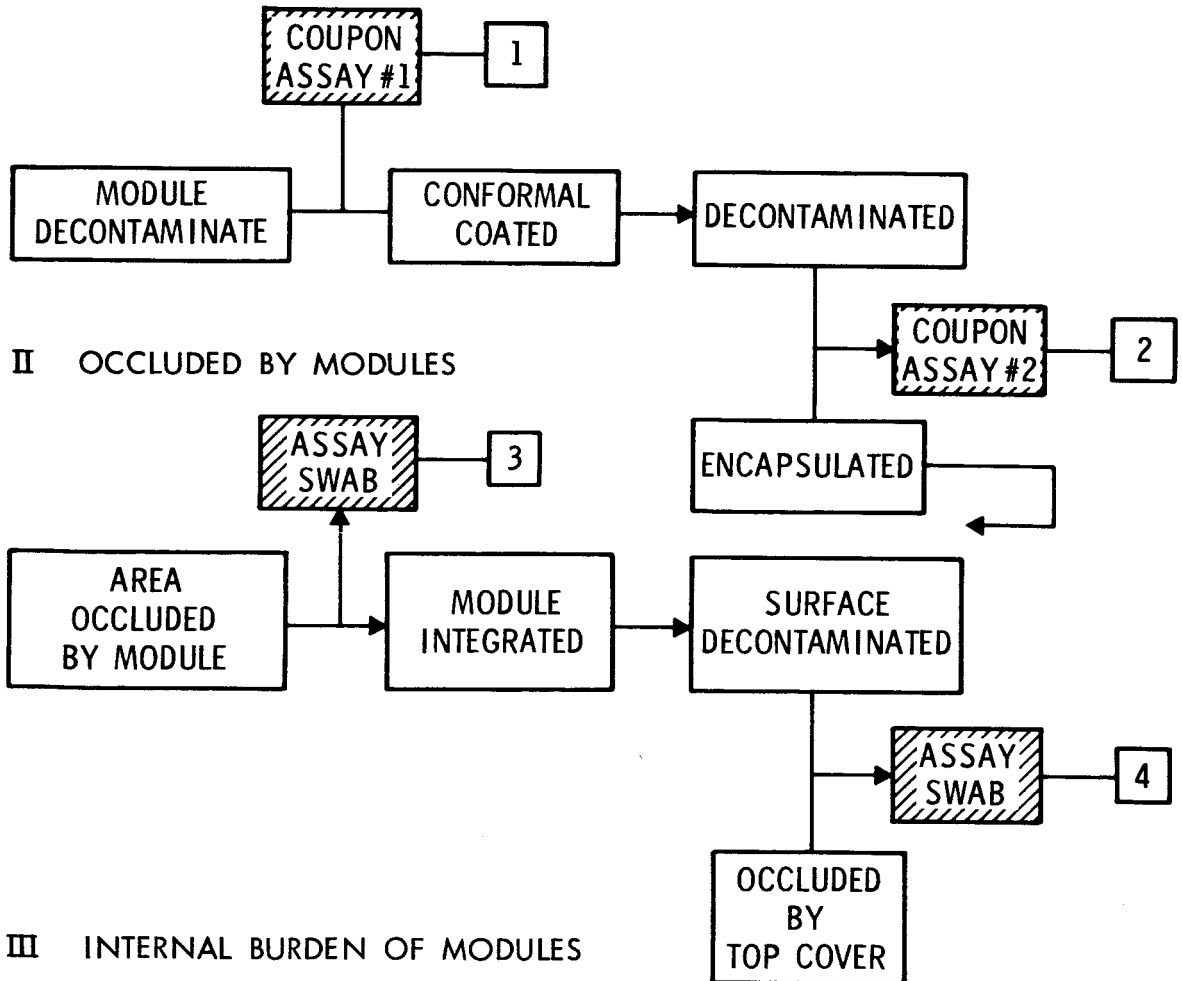


Figure 7.

INTERNAL AREAS OF SPACECRAFT

I OCCLUDED BY MATERIALS



III INTERNAL BURDEN OF MODULES

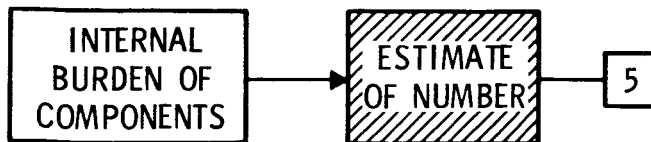
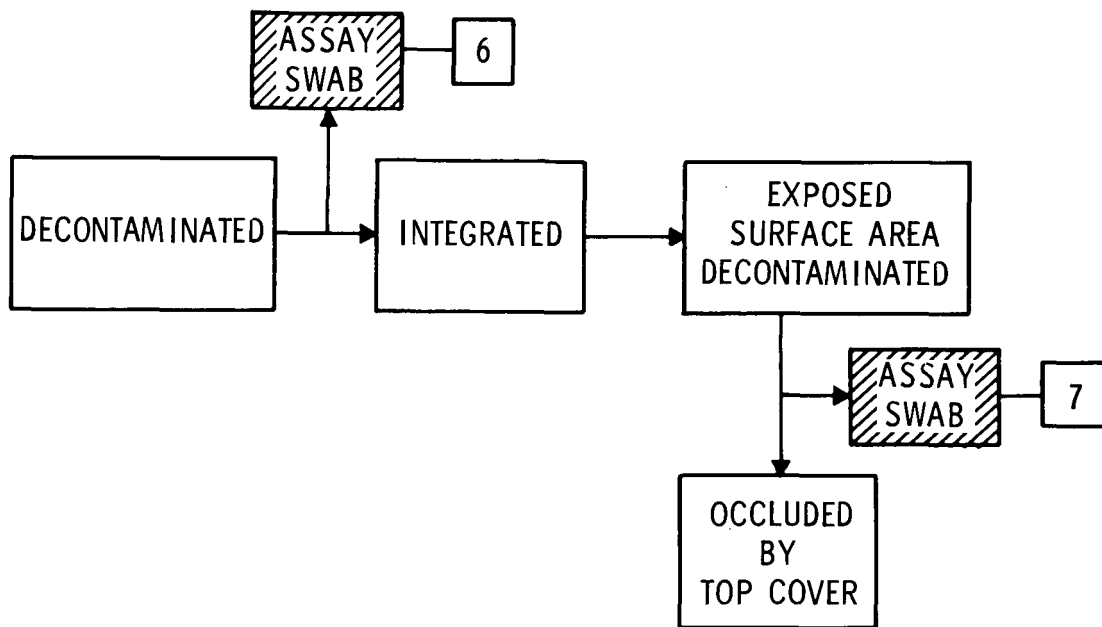


Figure 8.

INTERNAL AREAS OF SPACECRAFT

I BY ATTACHMENTS, INSTRUMENTS, ETC.



II EXPOSED SURFACE AREAS OF STRUCTURE

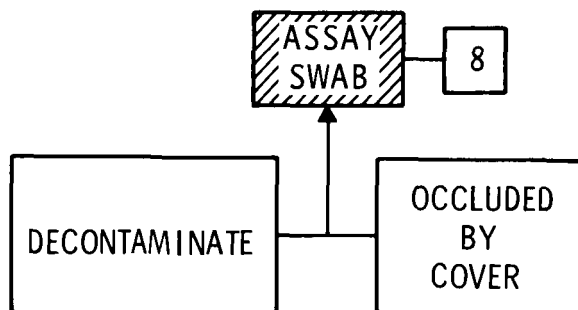
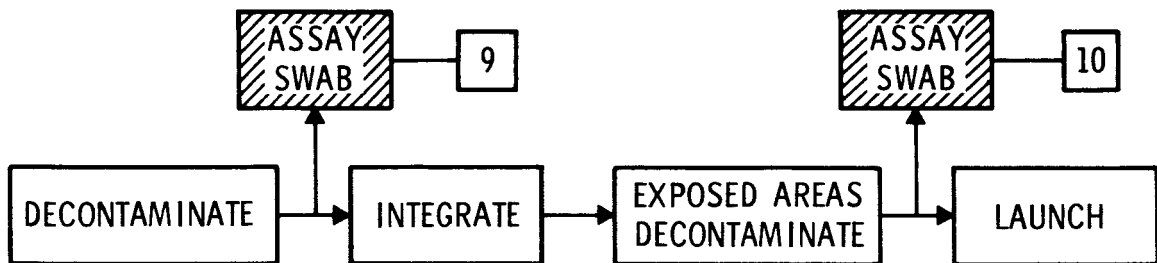


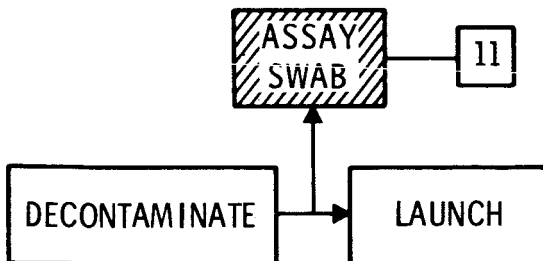
Figure 9.

EXTERIOR AREAS OF SPACECRAFT

I AREAS OCCLUDED BY ATTACHMENTS



II EXPOSED AREAS OF SPACECRAFT



III INTERNAL BURDEN



Figure 10.

The electronic modules were the first component parts of the spacecraft that were decontaminated. Decontamination was achieved by immersion in 90% isopropyl alcohol. The modules were agitated for at least three times during a 15 minute immersion. Control strip was decontaminated at the same time as the module. A coupon was taken off for assay. The electronic circuits were then conformal coated with a bacteriostatic epoxy. The circuit modules then went their route in the laboratory for electrical tests. Upon arriving back to the encapsulation area they were again decontaminated in the same manner as previously described, and one other decontaminated coupon was removed for assay. The circuit modules were then encapsulated. Prior to mechanically integrating the module into the spacecraft structure the area that would be occluded by the module was decontaminated. This area was then sampled by the microbiologist and assay performed. After module was integrated and just prior to its occlusion by the top cover all the exposed surfaces were decontaminated. Again samples were taken and assays performed. The internal burden of components were estimated and will be added to the total estimated viable count.

The areas that were to be occluded by an attachment instrument or structural member were first decontaminated. The areas decontaminated were then sampled and assays performed. The attachments were integrated, then the exposed surface area decontaminated. Samples for assays were then taken. In the same manner other exposed areas of the interior of the spacecraft were treated.

In a like manner the exterior areas of the spacecraft both occluded and exposed will be treated.

The decontamination was achieved by using—cotton swabs and lintless cotton cloth wipers that were first immersed in propanol.

The sampling of decontaminated areas for the assays, the assaying and colony counts were conducted by the Space Biology Branch at Goddard.

It is felt that the greatest source of contamination to a spacecraft will be from the technicians themselves and from the generation of debris that occurs during mechanical integration and assembly. It was therefore determined that clean room facilities should be built that would allow for aseptic:

- a. Decontamination of spacecraft components.
- b. Sampling of areas for assays.
- c. Mechanical integration.
- d. Final assembly.

Figure 11 is a view taken in the bio-clean area of the clean room complex, Mechanical Systems Branch, Goddard Space Flight Center. The technician is decontaminating the surface of a component that will be occluded by an attachment. All such areas are monitored for microbial contamination immediately to being occluded. The spacecraft was placed upon a dolly that was previously decontaminated and remained approximately one foot from the face of the air inlet filter during final decontamination and/or assembly. The personnel remained downstream of the spacecraft at all times. The tools required in assembly were first sterilized before being brought into the bio-clean area. They were brought into the area in sterile containers. All personnel who entered the bio-clean area wore lint free clean room garments that were sterilized and treated to eliminate static electricity. They also wore boots, hood, face mask and disposable gloves.

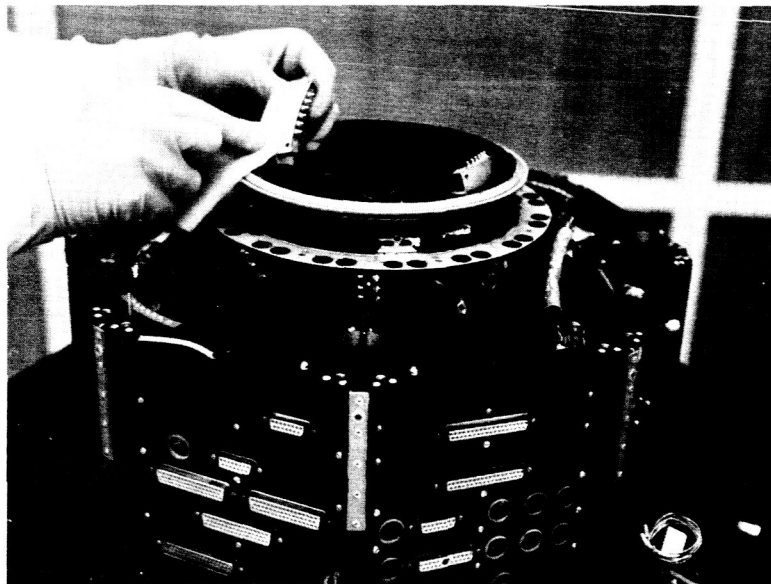


Figure 11.

Preliminary data pertaining to decontamination effort to date is shown in Figure 12. Data was furnished by the Space Biology Branch at Goddard.

MICROBIAL CONTAMINATION AIMP SPACECRAFT

ASSEMBLY PHASE	AVERAGE VIABLE PARTICLES PER SQ. FT.	
	BEFORE DECONTAMINATION	AFTER DECONTAMINATION
1	21,169	563
2	22,388	230
3	1,000	75
4	2,125	90
AVERAGE SQ. FT.	11,670	239
AVERAGE SPACECRAFT (500 SQ. FT.)	5,835,000	119,500

Figure 12.

The preliminary data is based upon contamination detected during the assembly of the spacecraft structure. It relates to those areas that were occluded by an attachment, support or another structural member. These preliminary data indicate that a significant reduction in microbial contamination on a spacecraft system can be obtained.

The manner in which the spacecraft is assembled in controlled areas with the final assembly, tests and decontamination being conducted under asepsis conditions in class 100 laminar flow clean rooms will result in a further reduction of the microbial load. This assumption is based upon a continual natural die-off of vegetative organisms that will occur during the elapsed time between sampling and time of launch.

The decontamination techniques summarized in this paper were applied at the Goddard Space Flight Center in biologically decontaminating the Anchored Interplanetary Monitoring Platform (AIMP Spacecraft). The techniques employed in decontaminating the spacecraft system and the decontaminates used were

compatible with spacecraft reliability factors and were in agreement with the Office of Planetary Quarantine, NASA Headquarters.

To my knowledge this is the first attempt made to measure microbiological contamination of an entire spacecraft system during the phases of mechanical integration and/or assembly. The knowledge gained and techniques developed in carrying out the decontamination program may be applicable in developing sterilization techniques for future interplanetary spacecraft and/or landing capsules.

The author gratefully acknowledges the assistance of:

Dr. Charles R. Phillips, Mr. Robert K. Hoffman and associates, U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland for conducting decontaminate kill effectiveness tests on control strips.

Mr. G. F. Mallison, Chief of Biophysics Section, Communicable Disease Center, Savannah, Georgia for suggesting small-pore filter to reduce spore contamination in alcohol bath.

Dr. Martin Favero, U.S. Department of Public Health, Communicable Disease Center, Phoenix, Arizona for conducting bacteriostatic tests on conformal coating.

Mr. Edmund Powers and Associates, Space Biology Branch, Goddard Space Flight Center for conducting biological assays and colony counts on spacecraft and hardware.

Dr. John Schutt, Stanford Ollendorf and Associates, Thermal Systems Branch, Goddard Space Flight Center for conducting tests to determine effects of decontaminates to thermal coatings.

Mr. William Grenier, Mechanical Systems Branch, Goddard Space Flight Center, for coordinating the materials and decontaminate compatibility tests.